ECOLOGY AND THERMAL INACTIVATION OF MICROBES IN AND ON INTERPLANETARY SPACE VEHICLE COMPONENTS

Thirty-eighth Quarterly Report of Progress

Order No. W-13411

July 1, 1974 - September 30, 1974

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Introduction

In the last quarterly report, the results demonstrated that microbial isolates from "fallout strip" experiments could be subcultured, grown, and harvested as spore crops and still maintain their heat resistance. The Cape Kennedy isolate 4-6 (Bacillus brevis) spores, in particular, was the most heat resistant of these three hardy spores examined.

In this quarter we continued our investigation in the following areas: (1) compared dry heat inactivation characteristics of 4-6 (B. brevis) spores and microbes from the Cincinnati soil samples at 105, 112, and 125 C; (2) characterized the survival curves of 4-6 (B. brevis) spores at 112, 115, 118, 120, and 125 C, and 1.2 μg of water per ml of headspace air (closed tin-can system); and (3) compared the morphological characteristics of 4-6 (B. brevis), 6-12 (B. lentus), 7-11 (B. coagulans), and B. subtilis var. niger spores by scanning electron microscopy.

I. EXPERIMENTAL

A. Dry heat oven experiments

Several experiments were conducted to determine the nature of the thermal inactivation curve of 4-6 (B. brevis)

spores and microbes from the Cincinnati soil samples at 105, 112, and 125 C. Cincinnati soil samples were weighed in 0.5-g amounts in stainless steel cups and dried in a 50-C oven for 1 hr prior to exposure at the desired temperature in a dry-heat oven. Also, 4-6 (B. brevis) spores stored in sterile double distilled water at 4 C were insonated for 24 min, diluted in double distilled water, and dispensed in 0.01-ml amounts in stainless steel cups with a repeating dispenser. These samples were also dried for 1 hr in a 50-C oven and then exposed at the appropriate temperature in a dry-heat oven. After exposure at appropriate time intervals, the cups containing either soil or B. brevis spores were removed from the oven. Sterile microbeads were placed in cups containing B. brevis spores, insonated in peptone water, and assayed for plate counts. Cups containing soil samples were also placed in peptone water, sonified, and assayed by the conventional plate count procedure, using TSA supplemented with 0.1% soluble starch and 0.2% yeast extract.

B. Heat resistance characteristics of 4-6 (B. brevis) spores at 112, 115, 118, 120, and 125 C, and 1.2 µg of water per ml of headspace air.

Using our standard procedure, a portion of spores stored in double distilled water at 4 C was insonated for 24 min. The insonated spores were diluted and a repeating

dispenser was used to deliver 0.01 ml into each stainless steel cup for a concentration of about 10^5 spores per cup. The cups were arranged on circular shelves and placed in 206 x 300 tin cans. Each shelf contained 30 cups, and each can contained four shelves for a total of 120 cups per can. The cans, lids, and contents were dried in a vacuum oven for 90 min at 45 to 50 C (at 1.5 in. Hg pressure absolute). To increase the drying rate, the oven was purged with dry nitrogen every 10 min for the first 70 min, followed by five consecutive purges of nitrogen, with a vacuum cycle between each purge. After the cans, lids, and contents were dried, they were removed from the oven and cooled to about 30 C in the equilibration hood. The cans, lids, and contents were kept in the equilibration hood for a minimum of 3 hr, after which they were sealed, in order that each can would contain 1.2 µg of water per ml of headspace air.

After the cans were sealed, they were heated in oil baths at 112, 115, 118, 120, and 125 C and exposed at various time intervals. The cans were removed from the oil bath and cooled in a refrigerated water bath. Spore survivors were assayed by the conventional plating method, using TSA supplemented with 0.2% yeast extract and 0.1% soluble starch.

C. Scanning electron micrographs of nonheat-treated spores

In this investigation four Bacillus species were examined. From a spore stock suspension, approximately 1.0×10^9 spores per ml were air dried through a 0.45-μ membrane millipore filter, and the pellet scraped with a sterile scalpel. This pellet was placed in a screw-cap vial and stored in a 4-C refrigerator before being shipped for scanning electron microscopy examination. Another portion of the spore suspension was mixed thoroughly in a 2% glutaraldehyde fixative, and placed in a 5-ml screw-cap vial.

Scanning electron microscopy services have been provided by Dr. P. S. Lin of the Radiobiology Division, Tufts University School of Medicine, Boston, Massachusetts.

II. RESULTS AND DISCUSSION

A. Dry heat oven experiments. Heat resistance of microorganisms in Cincinnati soil and of the Cape Kennedy spore isolate 4-6 (B. brevis).

The heat resistance of microorganisms in Cincinnati soil and spore isolate 4-6 (B. brevis) were determined at 105, 112, and 125 C in a dry-heat oven. The results are shown in Figures 1, 2, 3, and 4. In Figure 1, the survival curves show a rapid decline in the number of viable spores at the early stage of heating, followed by a gradual decline in the number of spores. When heat exposure was extended to 14 days (Fig. 2),

the magnitude of the initial loss in the number of spore survivors was still evident at all three temperatures. From the data shown in Figures 1 and 2, it is apparent that a soil sample consists of a population of several species of microorganisms. Therefore, when we extend the heat exposure at a certain temperature, we are actually heat selecting the microorganisms of varying heat resistances. Perhaps at some point in the heat process, we could achieve the heat selection to one spore species.

In Figure 3, the data show no log loss in the number of viable spores for the Cape Kennedy spore isolate 4-6 (B. brevis) at 105 and 112 C for over 56 hr. The survival curve at 125 C shows a "shoulder" followed by a steep decline in viable counts. Also, the tailing effect was noticeable at the latter part of the survival curve when spore survivors are around 10 per test unit. When the heat process was extended to 14 days (Fig. 4), the survival curves at all temperatures show "shoulders" at the early portion of the heating process.

Another interesting aspect in this study is the similarity in the survival curves of the Cape Kennedy spore isolate 4-6 (B. brevis) at 105 and 112 C (Fig. 4) to the portion of the survival curves of microorganisms in Cincinnati soil at 105 C and 112 C (Fig. 2).

B. Survival curves of Cape Kennedy isolate 4-6 (B. brevis) spores in a closed tin can system.

The dry-heat survival characteristics of the Cape Kennedy isolate 4-6 (B. brevis) spores was also determined in a closed tin-can system at several temperatures (112,115, 118, 120, and 125 C) and 1.2 µg of water per ml of headspace air.

The terminal sterilization process for the Viking lander is set at 111.7 C and 1.2 µg of water per ml of headspace air for 30 hr at lethality. All survival curves at all temperatures show significant number of viable spores (Fig. 5). These results suggest that the terminal sterilization cycle presently employed for the Viking lander spacedraft be readjusted.

C. Scanning electron microscopy of four Bacillus species.

We mentioned in the 36th quarterly report about the use of scanning electron microscopy as a tool for directly enumerating microorganisms and also for determining viability by morphological characteristics. We continued this study (Figs. 6 to 13) for the three hardy Cape Kennedy isolates [4-6 (B. brevis); 6-12 (B. lentus); 7-11 (B. coagulans)]; and the B. subtilis var. niger spores. The ellipsoidal appearance of Bacillus was observed for all four species examined.

III. PROJECTED RESEARCH FOR NEXT QUARTER

Dry-heat resistance studies will be continued on microorganisms in Cincinnati, Ohio; Cape Kennedy, Fla.; Pasadena, Calif.; and Colorado soil samples. Identification, based on biochemical activity of heat-survivor isolates following subculturing, will also be conducted.

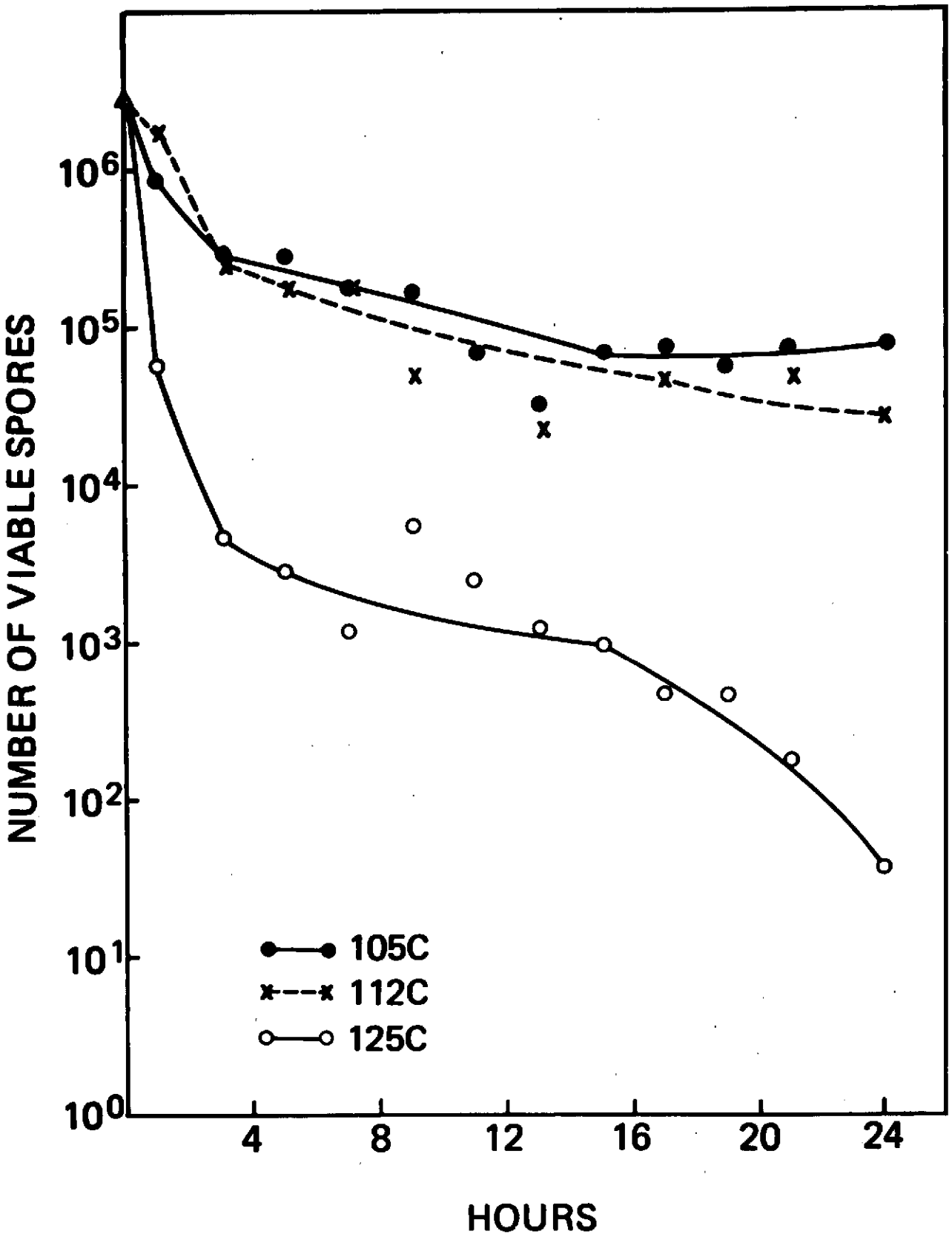

Fig. 1. Dry heat resistance of microorganisms in Cincinnati soil.

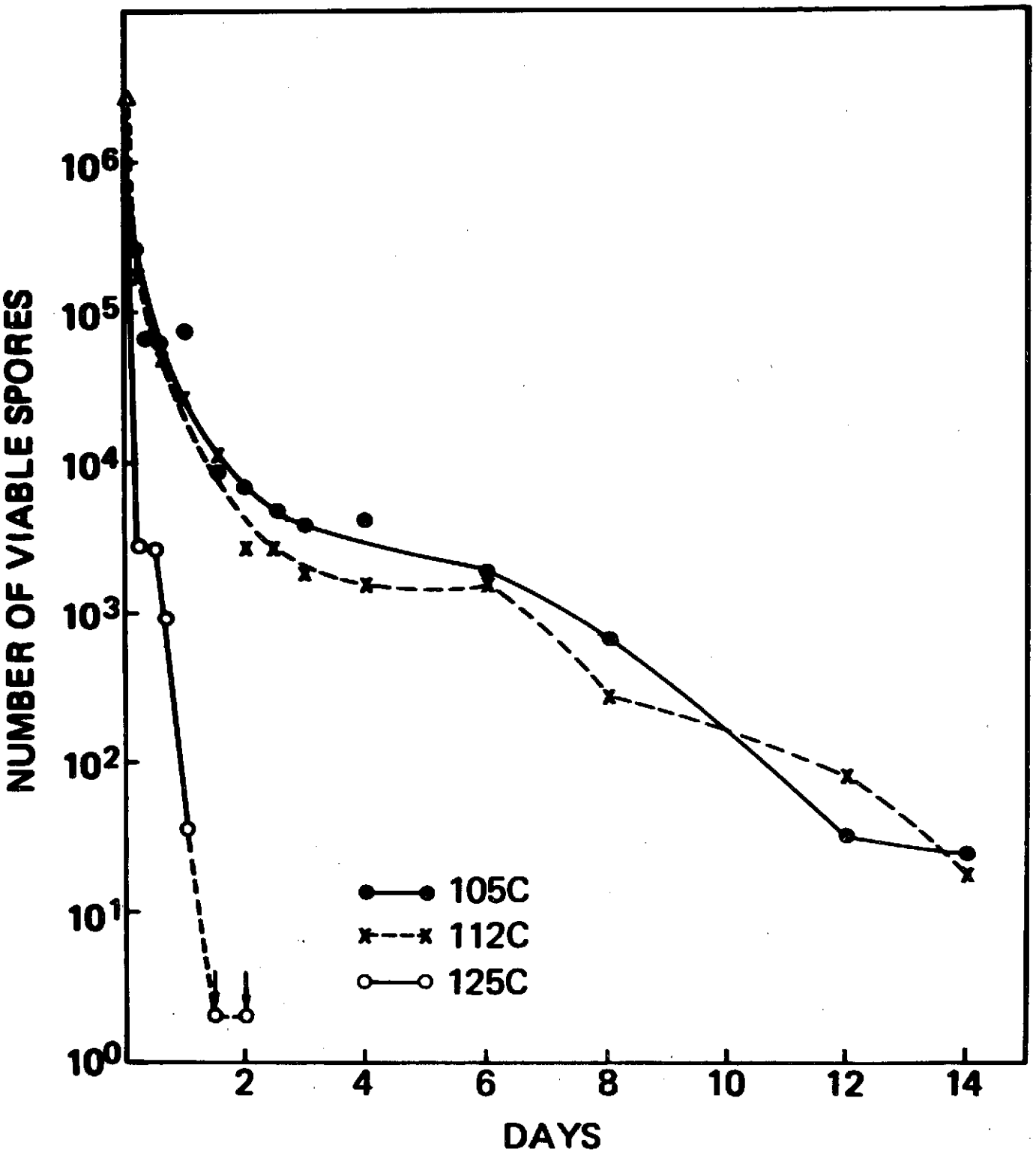

Fig. 2. Dry heat resistance of microorganisms in Cincinnati soil.

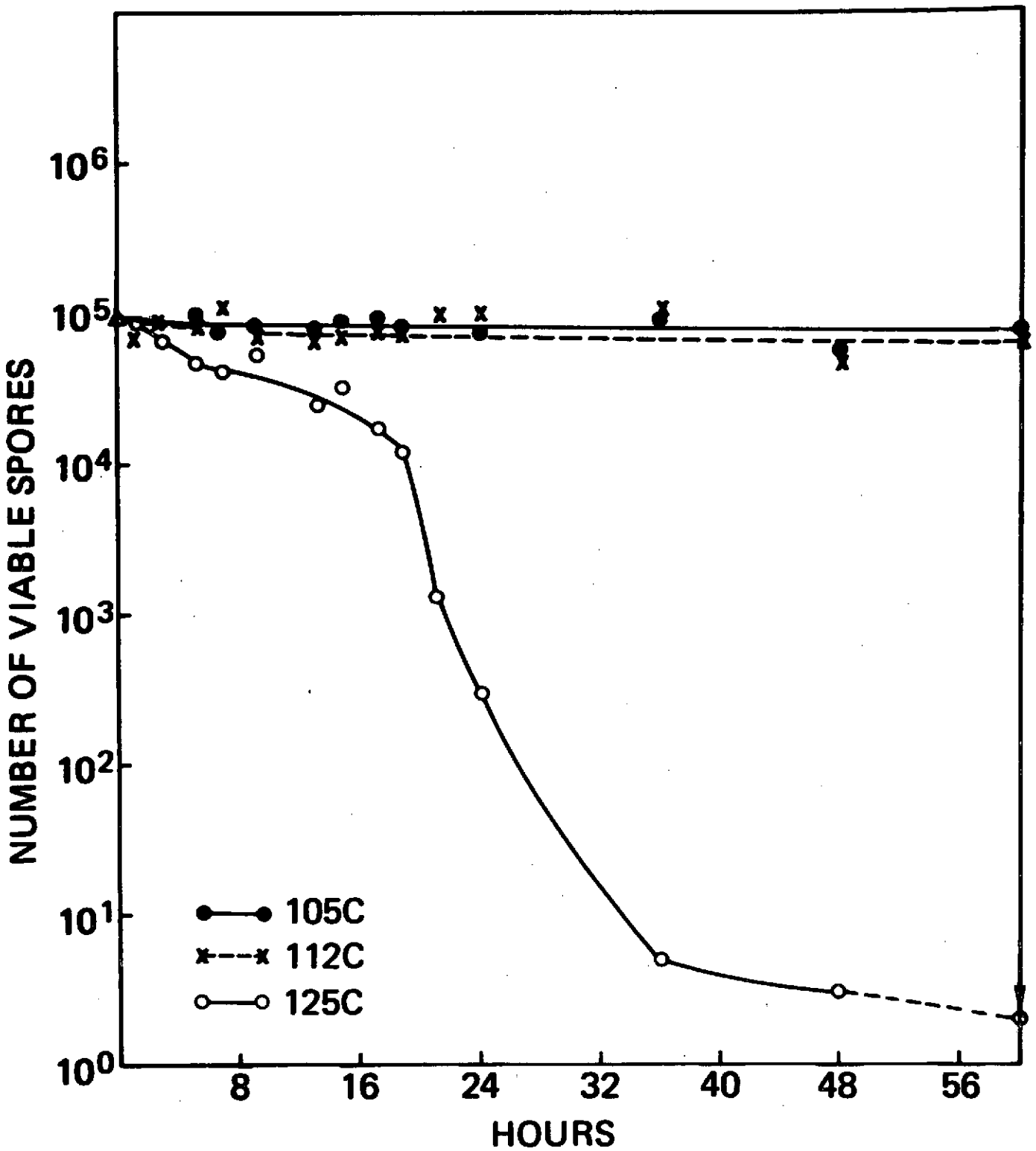

Fig. 3. Dry heat resistance of Cape Kennedy spore isolate 4-6 (B. brevis).

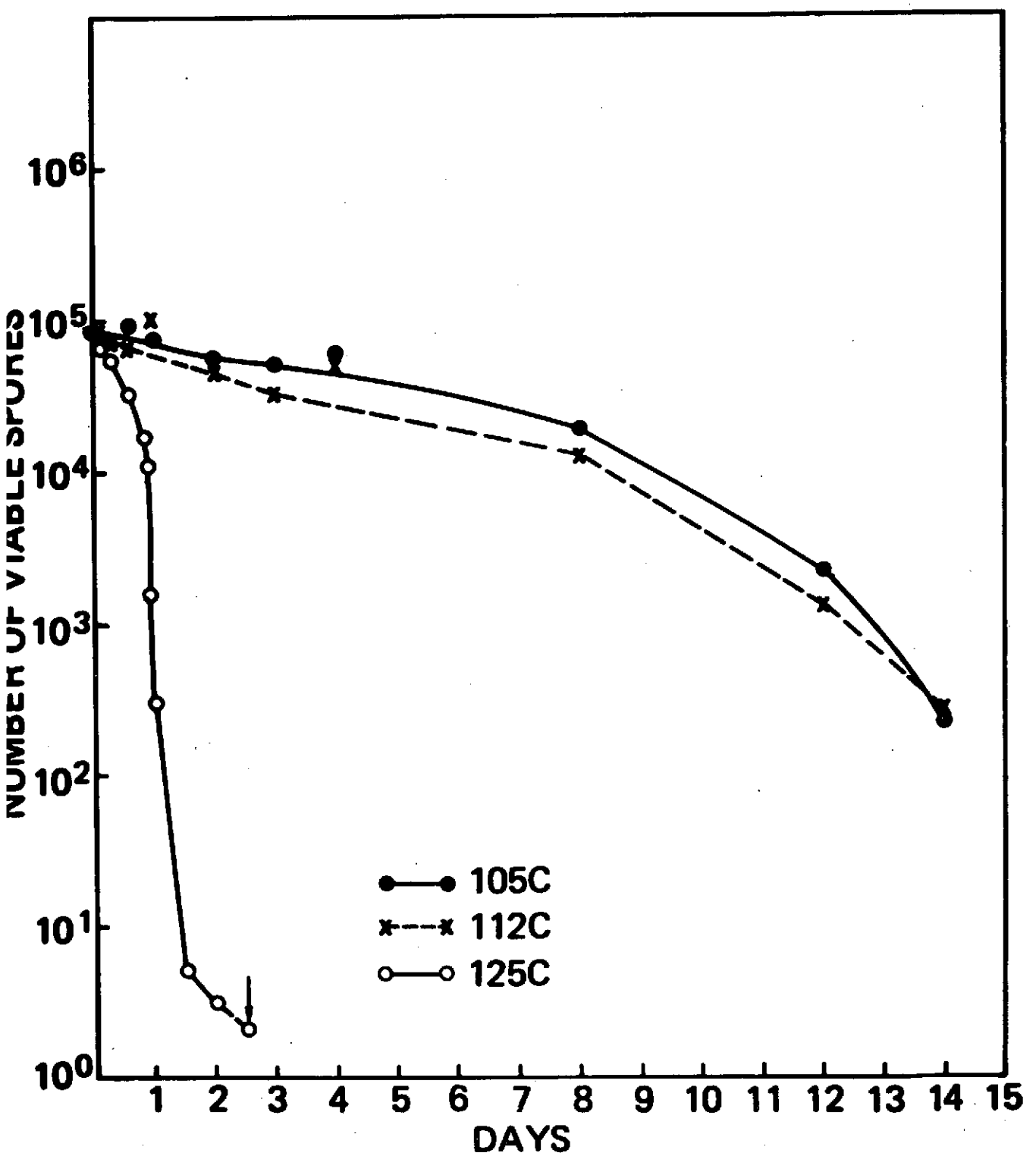

Fig. 4. Dry heat resistance of Cape Kennedy spore isolate 4-6 (B. brevis).

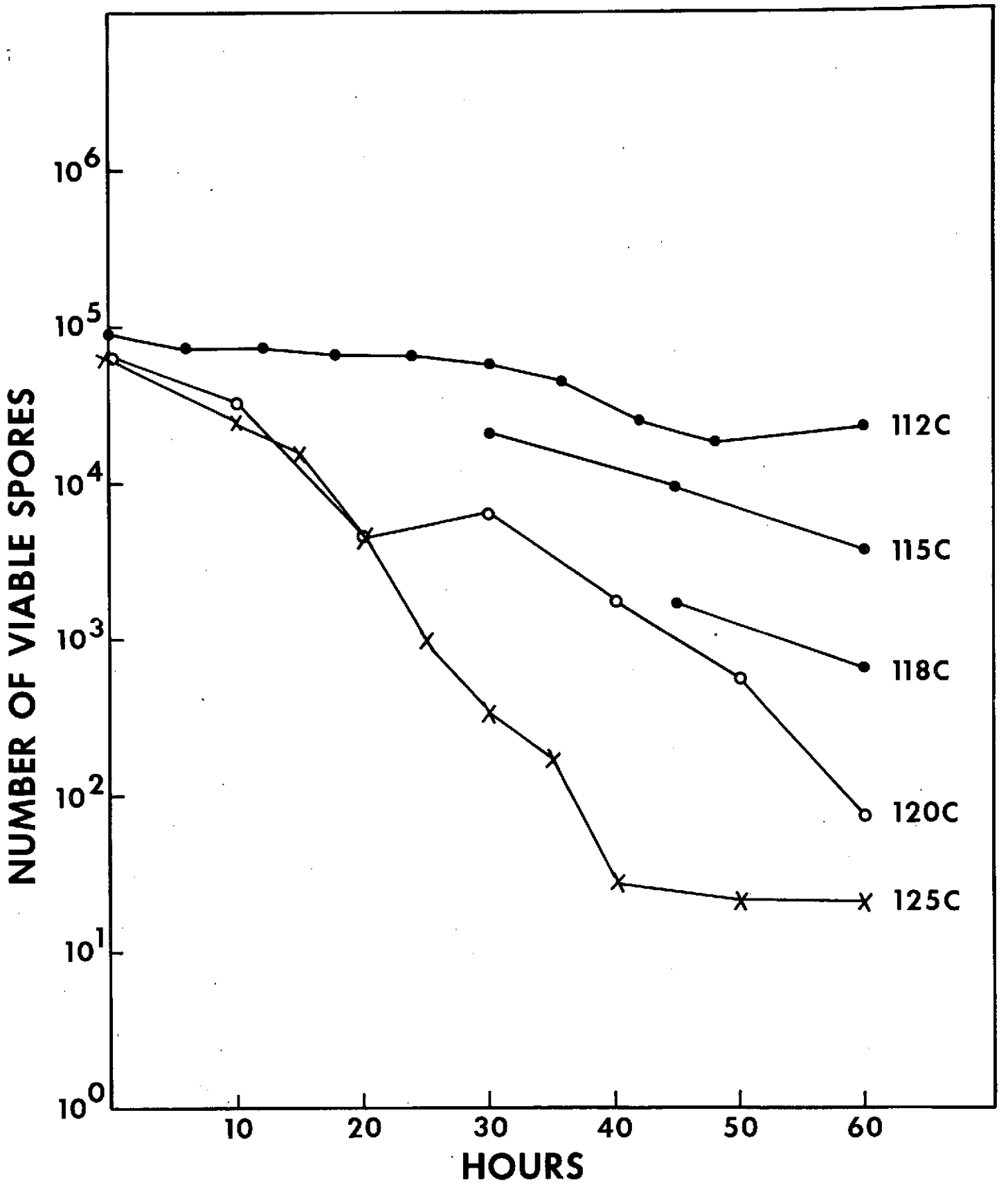

Fig. 5. **HEAT RESISTANCE CHARACTERISTICS OF 4-6 (B. BREVIS) SPORE.**

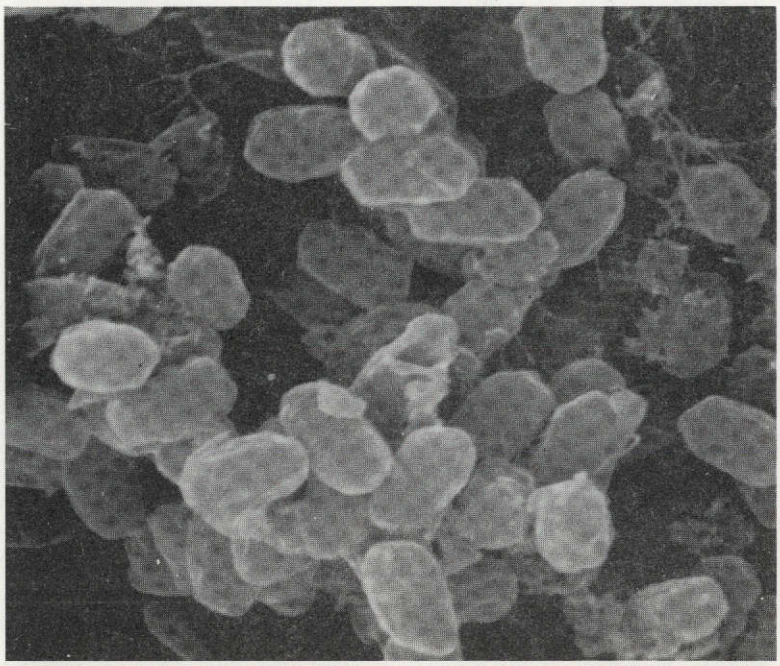

Fig. 6. Scanning electron micrograph of spores of B. subtilis var. niger. (Mag. 10,000 X).

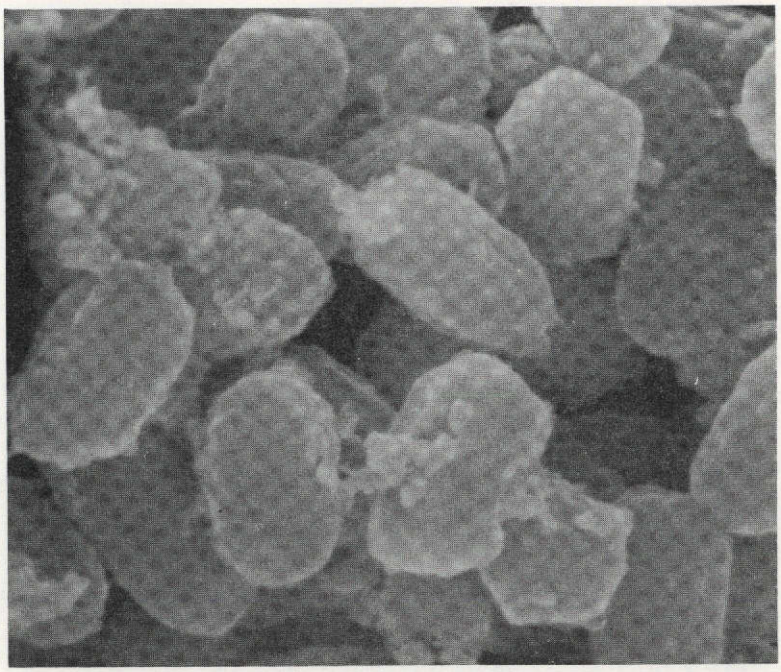

Fig. 7. Scanning electron micrograph of spores of B. subtilis var. niger. (Mag. 20,000 X).

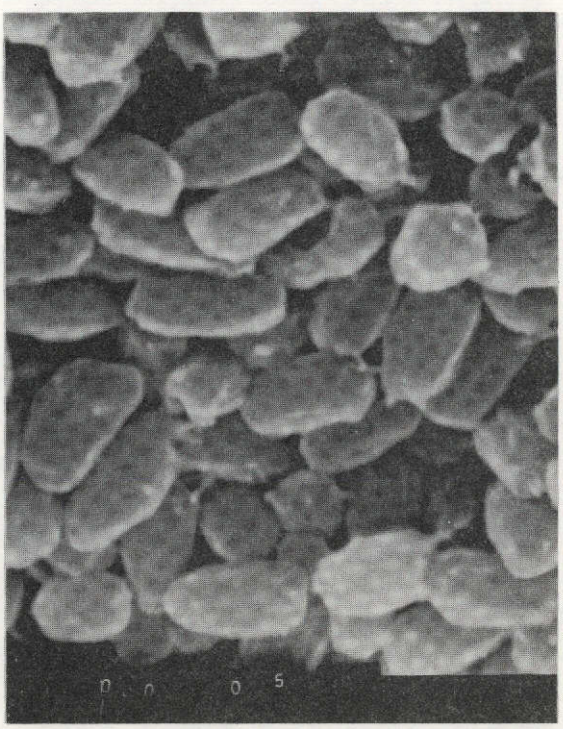

Fig. 8. Scanning electron micrograph of spores of 4-6 (B. brevis). (Mag. 10,000 X).

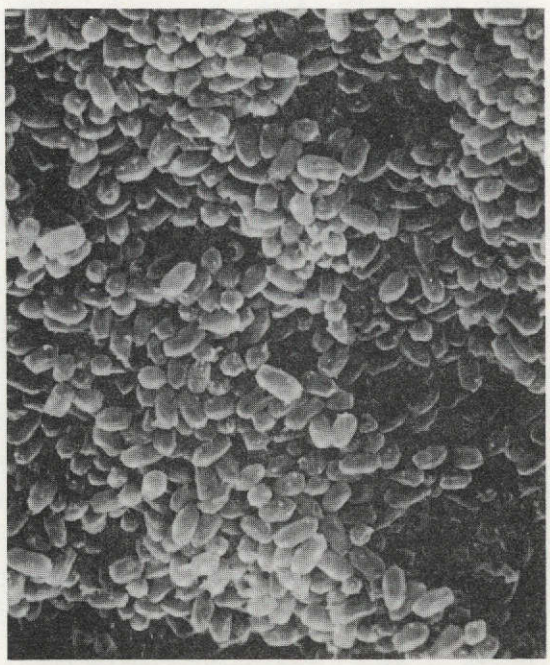

Fig. 9. Scanning electron micrograph of spores of 4-6 (B. brevis). (Mag. 3,000 X).

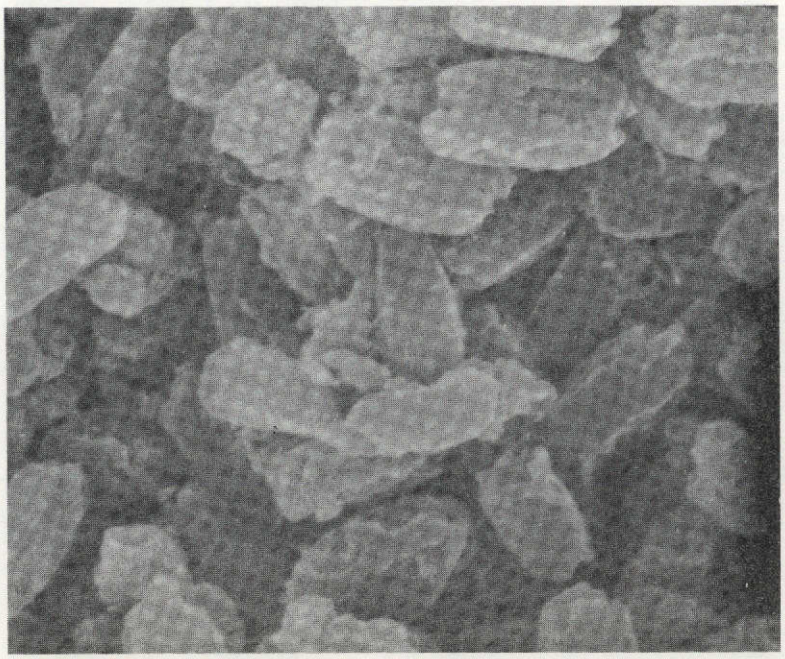

Fig. 10. Scanning electron micrograph of spores of 6-12 (B. lentus). (Mag. 10,000 X).

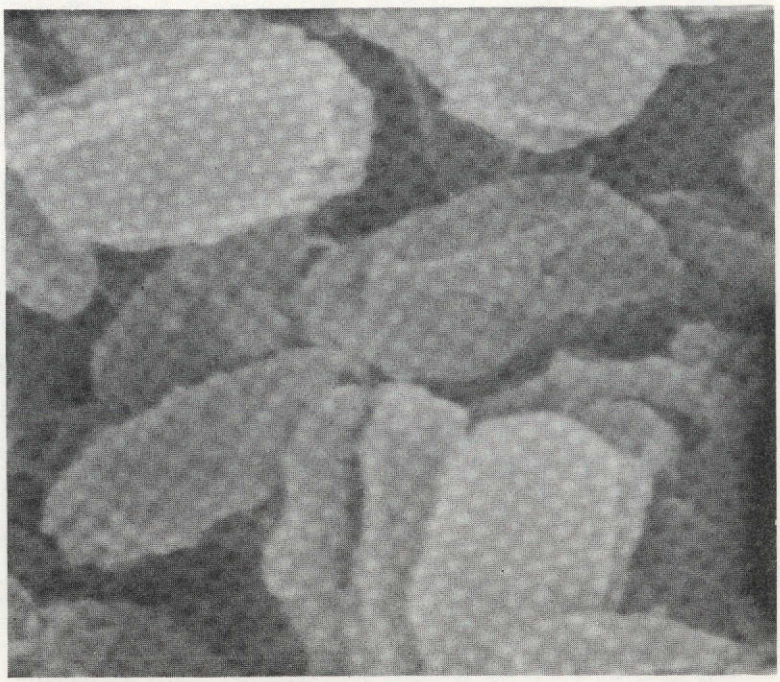

Fig. 11. Scanning electron micrograph of spores of 6-12 (B. lentus). (Mag. 20,000 X).

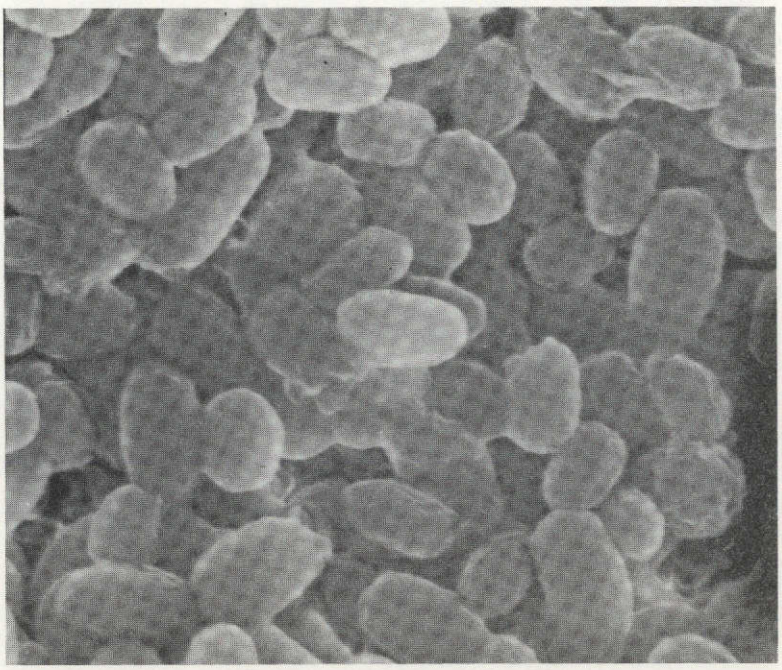

Fig. 12. Scanning electron micrograph of spores of 7-11 (B. coagulans). (Mag. 10,000 X).

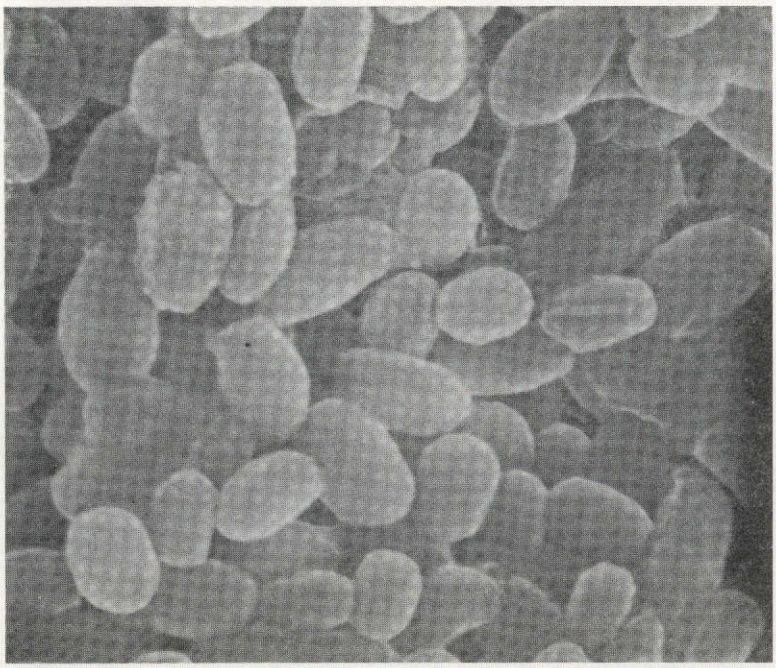

Fig. 13. Scanning electron micrograph of spores of 7-11 (B. coagulans). (Mag. 10,000 X).